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Figure 1. Project display at the Town pier providing information to public.

Overall Results

The project has experienced tremendous success over the past year including winning two prestigious awards: MassRecycle: "Municipal Innovation" and American Council of Engineering Companies: "Engineering Excellence" 2013 Silver Award. While prior work in this field has been undertaken from Louisiana to Great Bay, NH, this initial two acre demonstration project has achieved significantly higher oyster densities and reduced nitrogen 20% in the test area. Harbor wide application of the pilot results could produce one of the nation's first large scale TMDL compliance success stories; and attainment of the EPA "excellent" water quality classification by using aquaculture and salt marsh restoration; at 1/100th the cost of traditional alternatives. These two coastal habitats are the main consumers and recyclers of nitrogen in the environment as well as a critical link in addressing eutrophication from both anthropogenic and natural sources. At the same time, increased populations of oysters will lead to increased productivity and shellfish harvest as well as increased commercial finfish spawning habitat, increased protection from shoreline erosion and flooding, and buffering from ocean acidification.

Oyster Propagation Zone Monitoring

Intense monitoring was done during the primary growing season (June – October) when oysters are the most active, assimilating particles and nitrogen in the water. Compared with other commercially harvested shellfish, oysters have a higher capacity for growth under nitrogen-enriched and higher food supply conditions (nutrients) (Carmichael, 2012). Oysters store nitrogen (N) in tissue and remove nitrogen from the water through biogeochemical processes as they create natural habitats. In addition, through denitrification process, oysters remove nitrogen by converting unwanted nutrients into a harmless nitrogen gas -- the most common element in our atmosphere. It has been calculated that one million oysters removed 132 kgN in one year (Higgins, et al. 2001). Oysters have a high capacity for bioremediation (evidenced through millions of years of their presence as a keystone species in coastal habitats globally). Their population dynamic and carrying capacity in any given location depends on that location's environmental conditions. Oyster populations and salt marshes in Wellfleet Harbor, as well as globally, have been providing an amazing set of ecological services that have been declining and degrading in the last hundred years mainly due to habitat destruction, overfishing, pollution and other anthropogenic impacts (Table 1.) (Beck et al, 2011; Smith, 2011; Dibble et al, 2008; Fierra, 2007).

Through the process of water filtration oysters also remove particles like chlorophyll and suspended solids, decreasing turbidity and improving water clarity for subaquatic vegetation (Waldbusser et al, 2013). However, more recent oyster reef restoration in Chesapeake Bay showed exceptional results "*an acre of successfully restored oyster reef could remove 543 pounds (246 kg) of nitrogen in the Choptank River in just one year through denitrification. In addition, they found that the restored reef provides habitat for more than 24,000 organisms per square meter.*" (Kellogg, et al 2013) This restoration area had an average density of 131 oysters per square meter that were between 2 and 7 years old and a removal rate of 464 kg/million oysters, nearly 4 times the level found in Higgins. Much of this difference is attributed to obtaining more accurate measurements of the biogeochemical interactions between oysters and the co-dependent organisms. Kellogg et al. used a technique that captured as much of the benthos as possible to better replicate "real world" conditions for their measurements.

In Wellfleet, the project design is to go one step further and attempt to replicate prior laboratory scale findings with a field scale study which conducts measurements in situ and documents whether macro-scale water quality benefits can be quantified and verified.

Table 1. Salt marsh and Oyster ecological services.

Eco-services (type of 'functions')	**Salt marsh (one square meter)**	**Shellfish beds (one oyster)**
Nutrients/ Total nitrogen take	~ 21gN/m²/y	0.5gN/y
Carbon sequestration (pH buffer)	~ 210gCO_2/m²/y	42% dry weight soft tissue; and 11% shell
Sediment accretion	~ 1.3 cm/y (vertical accretion)	Sediment oxygenation
Water filtration, bioturbation, bioremediation	Coastal engineers	30-50 gallons/day natural water treatment plant

Wellfleet Harbor's oyster population has been declining since the mid-1800s, and based on historic data the current oyster population is largely cultivated aquaculture from hatchery stock and contains live oysters at densities that are less than 10% of past levels (Ebersol, 1882). There are essentially no natural oyster populations remaining and natural, co-dependent biodiversity has been severely diminished. Presently, the Town has been supporting cultching and propagation in Wellfleet Harbor in order to maintain a wild population while leased grants have maintained a viable commercial aquaculture industry. This project focuses on a two acre area at the confluence of Duck and Mayo Creeks, where several years of cultching prepared a suitable hard substrate for oyster spat to settle on and attach. The two acre project area with monitoring sites is shown at Fig 2; while the images showing the site before and after the cultch applications are presented at Figs 3 and 10.

In this case, oyster habitat restoration relies on local spawning brood stock to obtain millions of pelagic larvae that will settle on the provided cultch (clam substrate). The natural abundance of local wild seed lowers the restoration costs and helps maintain native oyster stock genetics which is believed to support more locally disease resistant oyster strains. In addition, if the population is allowed to grow for more than three years (based on current policy), the adult size would increase, increasing the spawning potential, biodiversity and water quality. **The water quality** and ecosystem health are the key impediments for both natural oyster propagation and aquaculture industry.

This project is meeting three goals that are interconnected and interdependent:

1. Providing healthy domestic/local seafood industry.
2. Providing critical coastal habitats with restored ecosystem functions.
3. Providing the water quality that will continue to support the above.

Monitoring results and water quality results are continued below.

Monitoring Schedule and Activities (May – December 2012):

1. Water samples were collected from a micro-grid of 7 sites as indicated below (Mayo Creek, Duck Bill, Channel, Transects 1-3-0, YSI probe; all done by the Center for Coastal Studies, Provincetown)
2. YSI probe for water quality was activated on June 15 2012.
3. Biodiversity assessment: counting common species, including predators/oyster drill (Table 2; Figs 4-5)
4. Oyster count, abundance, survival and density – counting all oysters (alive and dead 'boxes"), using the quadrat 0.25m²; manual counter and taking images (Table 3; Figs 6-7)
5. Oyster measurements for the groundtruthing data – using quadrat 1 m²; and images (Fig. 8)

Figure 2. Duck Creek project area with monitoring sites and transects.

Figure 3. Project area: left image is from May 2010; and the right image is from October 2012.
Total amount of cultch placed on the project site in two years was about 150 tons;

Table 2. Biodiversity monitoring data results.

Common species	Month 5-12	Transect # 1	2	3	4	5	0/control
Ribbed mussel (*Geukensia demissa)*	5	1					
	6	3				1	5
	7			1		1	
Mud crab (*Neopanopeus sayi)*	5	1	8	11			3
	6	27	17	15	32	8	16
	7	11	42	50	6	11	
	8	13	9	29	13	15	
	9	9		19			
	10		9			7	
	11	11					
Amphipods (*Gammarus*)	6	28		15	5	8	
	7	12	22	20		15	
	8	15	3	9		16	
	9	10		3			
Periwinkle (*Littorina littorea*)	5			4			
	6	1			3		10
	7		6			4	
Shrimps (*Palaemonetes* spp); high abundance in August and Sept. was captured on video camera	6			1	2	1	
	8		17	4	5	10	
	9	6					
Hard clams *(Marcernaria m.)*	5	6					
	6	5	1	6	2	4	1
	7	3	6	1		1	
	8		21	3			
	11	3					
Mud snails (*Ilyanassa obsolete)*	5	60	0				
	6					25	
	7	40		4		45	
Oyster drills *(Urosalpinx cinerea)*	5						1
	6	1					
	7		1	7			
	8			4			
Mud warms (*Polycheate*)	5		1	1			
	6	2		5	3		
	7	2	2	7	3		
	8			3	2		
	9			1			
Anemones (*Ascidia*)	5			1			
	6	2	2	9		45	15
	7	2	7	10	1	2	
	8	2	4	5	4	8	
Sea lettuce (*Ulva sp*) % No presence in the rest of the season	5	10	0	0	0	0	0
	6	80	45	10	25	20	

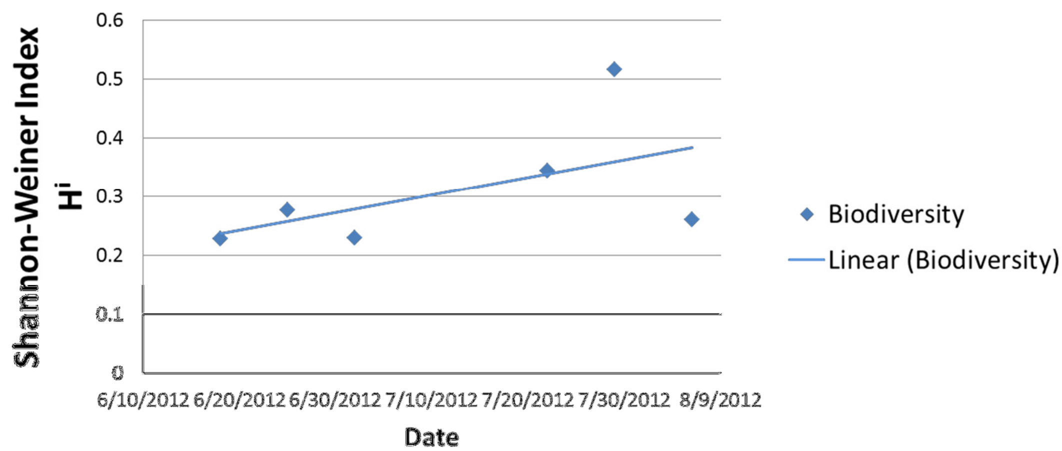

Figure 4. Diversity Index (H') – relative biodiversity abundance in the project site was the highest between June and August 2012.

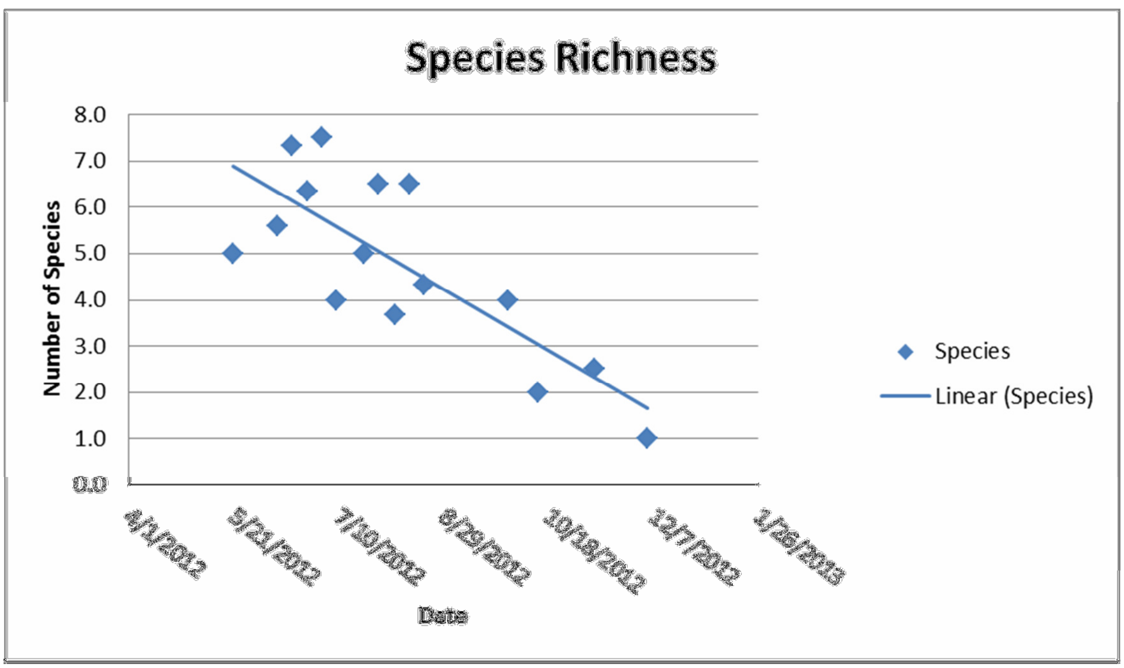

Figure 5. Species richness on the project site assessed between May and December 2012 was the largest during the summer months of June, July and August.

Table 3. Oyster relative abundance by transect (quadrants).

Transect area #	Month/monitoring times #	Oyster # per 0.25m²	Oyster # per m²	Box # per 0.25m²	Box # per m²
1	5/1	66	512	23	46
	6/3	196		22	
	7/3	321		18	
	8/1	382		26	
	9/1	214		13	
	11/1	356		36	
2	5/1	300	681	47	66
	6/3	251		33	
	7/3	345		26	
	8/2	408		26	
	10/1	398		32	
3	5/1	473	844	37	60
	6/3	326		31	
	7/3	426		31	
	8/1	416		21	
	9/1	468		31	
4	5/1	379	741	22	108
	6/2	304		57	
	7/1	299		83	
	8/1	499		53	
5	6/2	244	512	48	64
	7/2	192		34	
	8/2	203		33	
	10/1	271		18	
	12/1	371		28	
Relative ABUNDANCE/m²			**658**		**69**
0 (control site, outside the 2 acre project area)	5/2	439	969	49	98
	8/1	342		45	
	12/1	673		53	

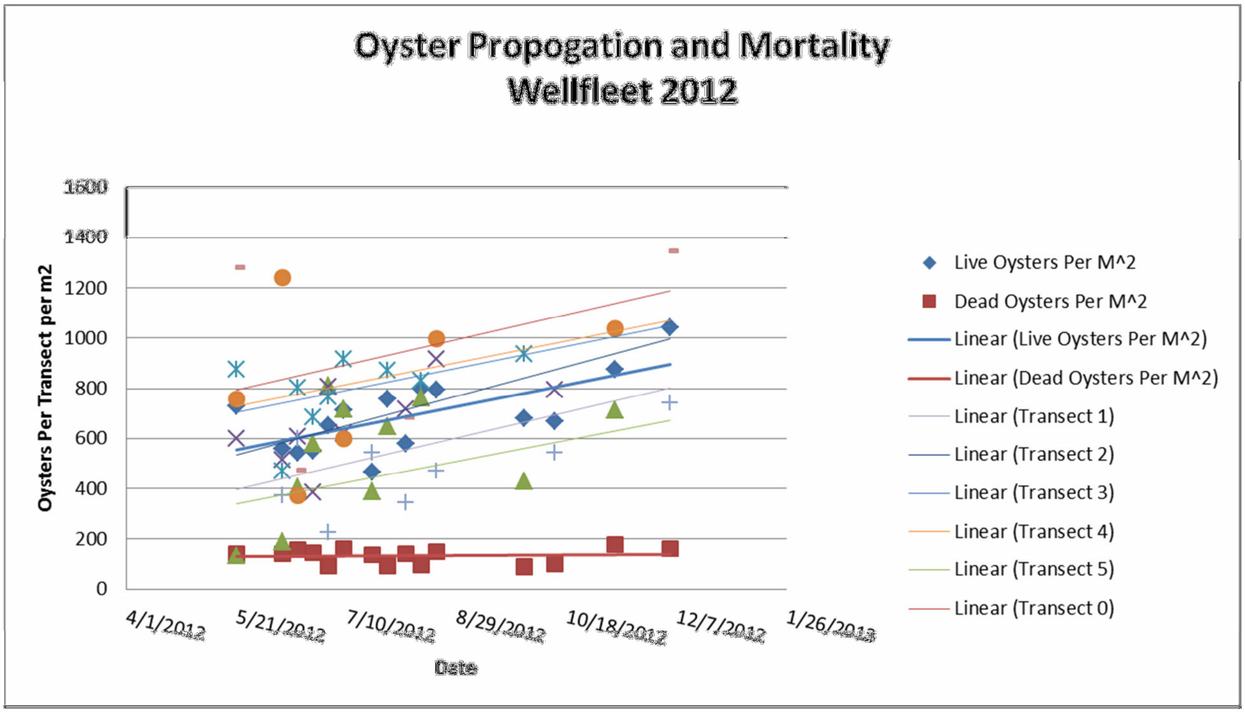

Figure 6. Shows all the transect data of live and dead oysters per square meter/per transect.

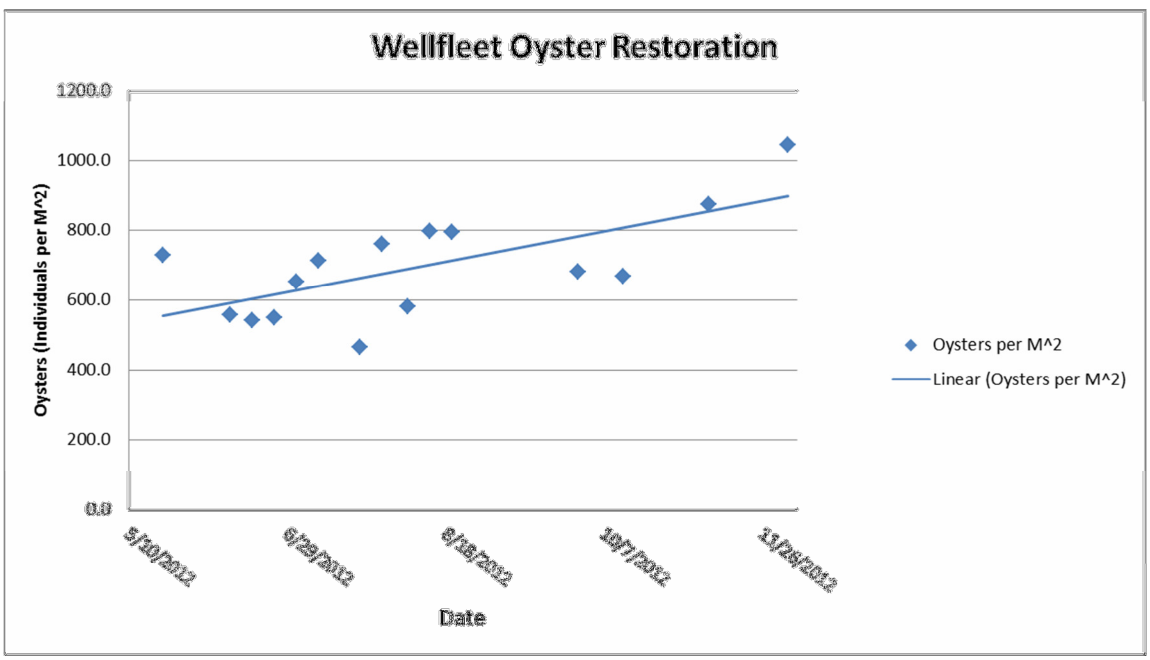

Figure 7. Relative abundance of oysters at the project site between May-December 2012.

Based on this season's monitoring results, the current oyster abundance in the two acre (8,094 m²) project area is approximately 4 million oysters, about 494 oysters/m². In 2011 our monitoring results estimated oyster abundance to be at 2 million oysters in the project area. Therefore, we are estimating that in the next two years we are going to achieve the project's goal of 8 million oysters within the project area.

Figure 8. One square meter quadrant was used for the groundtruthing purpose (side scan sonar benthic assessment), the quadrat accounted for 249 oysters, with the average size of 2.1 cm;

GPS coordinates for one square meter quadrant:

414858.423	4642618.410	-0.763	wlhbr-01
414858.608	4642619.471	-0.750	wlhbr-02
414857.518	4642619.609	-0.757	wlhbr-03
414857.382	4642618.620	-0.748	wlhbr-04

Project Task #4" Establishing a Target Oyster Population for Duck Creek:
How many oysters do we need to improve the nitrogen content in the Duck Creek area?

Duck Creek comprises an area of about 50 acres and a water volume of about 550m x 350m x 2m at high tide. Tidal range is quite large with the estimated bathymetry at high tide of approximately 2m, going to zero pretty quickly exposing a large mud flat at low tide. On average, we expect that an actual volume of water in this area is about 385,000 cubic meters (100 million gallons) or 50-100 million if we use a range that includes the lower depth. Therefore, based on the nitrogen load in this area (Fig. 9), a population of 8 million oysters in the Duck Creek basin is a reasonable target of an adaptive management strategy to achieve sustainability and water quality goals, by providing a 'nitrogen sink' of about 4,000kgN/year (8,800 lbs). Based on adult oyster filtration rates of 50 gallons per day, this would further provide the capacity to filter the entire water volume perhaps as much as once per day, a level noted as consistent with excellent water quality from historic studies in Chesapeake Bay. In addition, at this population, productivity would increase dramatically due to the much larger number of resident spawning oysters. Those numbers are similar to the entire commercial harvest reported in Wellfleet for 2007. However, by establishing and maintaining this pilot area as an oyster propagation zone without harvest, it would provide missing ecoservices and improve water quality to meet the state and federal requirements while increasing the net harvest. Along with additional cultching of adjacent areas, this location could produce by itself a sustainable commercial harvest of at least a million oysters per year; supporting biodiversity, ecoservices (shoreline protection, fish habitat), and genetic diversity. The exact magnitude of benefits needs to be closely assessed and monitored over the next few years while the project efforts are aiming to establish the optimal minimum necessary oyster population to achieve these goals. Another important feature is that we currently don't have any large populations where specimens can grow beyond the harvesting size (3 inches), which is typically less than a 2 year old oyster. Having a closed propagation area would allow oysters to grow beyond five years which would support resident population by experiencing the cyclical die-offs which is believed to support disease resistance and maintain larger and more productive females (Smith, 2012). This approach is essential for maintaining wild oyster genetic diversity in the harbor, and providing critical habitats for juvenile fish and other species.

Figure 9. Total nitrogen measurements in Duck Creek in 2012 (source: Amy Costa), results show about 20% nitrogen reduction from 2011-2012 in the oyster propagation area and excellent water quality due to nitrogen sinking from the high oyster density.

<u>Project Task # 6: Sea lettuce and algae removal</u> – During the monitoring season 2011, in the late August and during September 2011, we noticed that the project site area was heavily covered *Ulva latuca* (sea lettuce) a classic sign of excessive nutrients. During the low tide the green mass of sea lettuce was lying on the cultch and already settled oysters, and during the high tide it was dispersed in the water column above the project site severely restricting light penetration. Our assumption was that removal would enhance oyster survival, new settlements and reduce nutrient loading. However, during this monitoring season the site didn't experience the same algal bloom as in the previous year (Fig. 10). Since the water quality results (Fig. 9) show more than 20% reduction in nitrogen between 2011 and 2012, our working hypothesis is that the nutrient reduction from oysters may have already impacted water quality enough to reduce the *Ulva* growth. It will be quite interesting to see if the *Ulva* does or does not reappear in season 2013. Anecdotally, we have seen a reduction in loose sediment commonly call a "black mayonnaise", which is the result of organic material decomposition (such as *Ulva*), and with increased number of oysters we hope to gather research data to assess and track this trend.

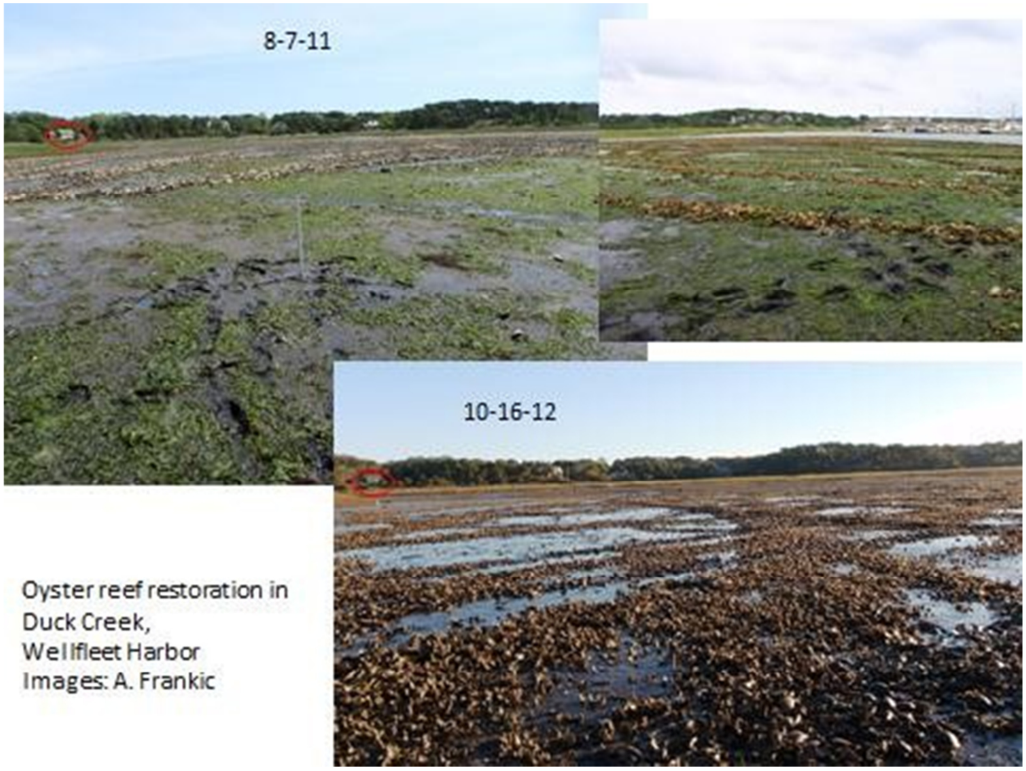

Figure 10. Before and after cultching and oyster propagation.

Next steps:
Our next season's monitoring practices will include underwater video camera so that we can capture biodiversity in the water column during high tides and not just at low tides. The biodiversity increase was noticeable in just one year after establishing the cultch substrate for oysters to settle on and grow. In addition, we noticed that monitoring practices doing transects can physically impact the clutched area and young oysters, as we have to sometimes walk on the site between transects (although whenever possible kayaks were used to minimize physical impact on the project site). Therefore, we are planning to establish a permanent wooden platform fixed on the bottom, from where we would do the monitoring assessments, attach cameras and other necessary equipment for water quality measurements. We are also going to establish two permanent transects, one square meter each, similar to the one on Fig 8. This will help us measure the oyster growth rate and support the groundtruthing process in the project site as well as provide comparisons with various commercial sites in the harbor.

In order to address the restoration and propagation activity in the whole of Wellfleet Harbor, we have received Conservation Commission approval to continue placing cultch at suitable sites (Fig.11). Based on the results from the side scan sonar and ground truthing at the project site, we will be able to better understand and assess the present distribution of wild oyster populations and provide estimates of the oyster size and density.

Therefore, our research objectives are to identify:
1) Population Count (starting with the project area as a test-bed),
2) Map of the extent of wild oyster beds in the harbor, and
3) Overall oyster population assessment.

In addition, we are going to take sediment core samples at the project site and throughout the harbor in order to start addressing research questions related to the historic assessment and distribution of different coastal habitats: oyster populations, shellfish beds, salt marsh and eel grass beds. These types of data will be important for future research projects to better understand the status and trend of the coastal habitats and how might they change due to human and natural impacts. They will also allow us to model historic nitrogen sinking and water quality benefits in order to better understand what extent of natural restoration can restore nutrient balance and achieve EPA designated excellent water quality throughout the harbor.

Figure 11. Current and planned areas for cultching and oyster propagation in Wellfleet Harbor. (map by A. Frankic)